REMARKS

Amendment to the Specification

The description of Figure 12, in the Brief Description of the Drawings, has been amended to include the sequence identifier for the amino acid sequence of human CCR1 as designated in the Sequence Listing. No new matter has been added.

Amendments to the Claims

The Claims have been amended to use consistent terminology and to delete reference to "an effective amount." No new matter has been added.

Objection to the Drawings Under 37 C.F.R. §1.821(d)

The Examiner has objected to the drawings in the instant application for failure to comply with 37 C.F.R. §1.821(d), which requires that a reference to a particular sequence identifier (SEQ ID NO:) be made in the specification and claims wherever a reference is made to a sequence. Specifically, the Examiner states that Figure 12 of the instant application describes an amino acid sequence without employing a sequence identifier either in the drawing or in the Brief Description of the Drawings.

The description of Figure 12, in the Brief Description of the Drawings, has been amended to reference the sequence identifier SEQ ID NO: 1, which is the sequence designation for the amino acid sequence of human CCR1 shown in Figure 12 as listed in the Sequence Listing for the instant application. Reconsideration and withdrawal of the objection are respectfully requested.

Rejection of Claims 1-163 Under 35 U.S.C. §112, First Paragraph

Claims 1 to 163 are rejected under 35 U.S.C. §112, first paragraph. The Examiner states that Claims 1-163 contain subject matter which was not described in the application in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner states that the Specification does not provide the guidance needed to predictably administer an anti-CCR1 antibody to an organism for clinical effect. The Examiner further states that the Specification

appears to be relying on procedures "well known to the skilled artisan" to provide the details of the claimed method and alleges that the administration of antibodies for clinical effect does not appear to have been well known to the skilled artisan at the time the application was filed. Claims 98-163 have been cancelled herein.

Whether the Specification would have been enabling as of the filing date involves consideration of the nature of the invention, the state of the prior art, and the level of skill in the art. MPEP §2164.05(a). The state of the prior art is what one skilled in the art would have known, at the time the application was filed, about the subject matter to which the claimed invention pertains. The relative skill of those in the art refers to the skill of those in the art in relation to the subject matter to which the claimed invention pertains at the time the application was filed. 35 U.S.C. §112 requires the Specification to be enabling only to a person skilled in the art to which it pertains or with which it is most nearly connected. Indeed, the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled in the art and already available to the public. *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991).

Applicants are submitting a Declaration Under 37 C.F.R. §1.132 by Dominic Picarella concurrently herewith as evidence that it was routine and would not have required undue experimentation for one of skill in the art to determine the route and duration of administration and the quantity of antibody or antigen-binding fragment to be administered to effect the methods claimed in Claims 1-97 at the effective filing date of the above-identified application. Dr. Picarella states that it was well within the ability of the skilled artisan to administer an anti-CCR1 antibody or antigen-binding fragment thereof as disclosed in the above-identified application and recited in the claims to inhibit a CCR1-mediated effect, *i.e.*, to inhibit interaction of a cell bearing CCR1 with a ligand of CCR1, to inhibit a function associated with binding of a chemokine to a mammalian CCR1, or to inhibit leukocyte trafficking, without undue experimentation.

In particular, Dr. Picarella states that at the effective filing date of the application, a large number of monoclonal antibody therapeutics were in clinical use and preclinical trials, as evidenced by Reichert, *Nature Biotechnology 19*:819-822 (2001) attached to the Declaration as Exhibit B. Reichert provides a review of various aspects of the clinical development of antibody

products and notes that between 1980 and 2000, 227 monoclonal antibody products entered commercially sponsored clinical studies. Thus, there was a large body of knowledge available to the skilled artisan in the field of antibody technology, particularly related to the formulation and optimization of antibody products for *in vivo* use, at the effective filing date of the application (Declaration, ¶4).

In addition, Dr. Picarella states that, given the disclosure of the application and the knowledge of the skilled artisan at the effective filing date of the application, it would have required only routine experimentation for the skilled artisan to make and use the claimed invention. In particular, Dr. Picarella states that the skilled artisan would have determined the pharmacodynamics profile *in vitro* (*i.e.*, the amount of antibody needed to completely inhibit, *e.g.*, chemotaxis, and the amount of receptor saturation necessary to achieve complete inhibition) and *in vivo* (*i.e.*, the dose of antibody to be administered to achieve *in vivo* the level of receptor saturation determined *in vitro*). The skilled artisan would also have determined the pK profile (*i.e.*, the concentration of antibody in the blood over time resulting from various amounts of administered antibody) of the antibody. These routine determinations would have allowed one of ordinary skill to make and use the invention commensurate with the current claims. Dr. Picarella also states that the procedures for determining both the pharmacodynamics profile and the pK profile were routine in the art at the effective filing date of the application and would not have required undue experimentation. (Declaration, ¶¶ 5-7).

Thus, Dr. Picarella states that it was routine and would not have required undue experimentation for one of skill in the art to determine the route and duration of administration and the quantity of antibody or antigen-binding fragment to be administered to effect the methods claimed in Claims 1-97 at the effective filing date of the application. Dr. Picarella also states that it was well within the ability of the skilled artisan to administer an anti-CCR1 antibody or antigen-binding fragment thereof as disclosed in the application and recited in the claims to inhibit a CCR1-mediated effect, *i.e.*, to inhibit interaction of a cell bearing CCR1 with a ligand of CCR1, to inhibit a function associated with binding of a chemokine to a mammalian CCR1, or to inhibit leukocyte trafficking, without undue experimentation (Declaration, ¶8).

Accordingly, as supported by the Reichert reference and the Declaration, the Specification was indeed enabling to a person skilled in the art to which it pertains or with which

it is most nearly connected for the invention claimed in Claims 1-97 at the effective filing date of the application. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 8, 13-15, 17-18, 20-35, 39, 44-48, 51-64, 70, 75-77, 79-80, 82-97, 103, 108-110, 112-113, 115-130, 136, 141-143, 145-146, 148-163 Under 35 U.S.C. §112, First Paragraph

Claims 8, 13-15, 17-18, 20-35, 39, 44-48, 51-64, 70, 75-77, 79-80, 82-97, 103, 108-110, 112-113, 115-130, 136, 141-143, 145-146, 148-163 are rejected under 35 U.S.C. §112, first paragraph. The Examiner states that these claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner states that these claims expressly require the biological material recited therein as 2D4, and thus, Applicants, their assignee or their agent must provide a biological deposit declaration.

Applicants are submitting concurrently herewith a Statement Under 37 C.F.R. §1.806 and §1.808 which states that murine hybridoma 2D4 has been accepted for deposit under the Budapest Treaty and that, except as permitted by 37 C.F.R. §1.808(b), all restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent. Applicants also note that the Specification provides the accession number, date of deposit, name and address of the depository, and taxonomic description of the deposited biological material (see, for example, page 18, lines 23-27). Thus, Applicants believe that the requirements of 35 U.S.C. §112, first paragraph, have been met. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 1-163 Under 35 U.S.C. §112, Second Paragraph

Claims 1-163 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner states that Claims 1-163 are vague and indefinite in the recitation of "an effective amount of an antibody" because one would reasonably expect that even a single antibody molecule would be inhibitory to a minute extent. The Examiner further states that it is unclear how the scope of claim 1 would be altered by the deletion of the limitation.

Claims 98-163 have been cancelled. In order to expedite prosecution, the remaining claims have been amended to delete reference to "an effective amount." This amendment does not narrow the scope of the claimed subject matter, as supported by the Examiner's statements. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Lisa M. Treannie

Lisa M. Treannie
Registration No. 41,368
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
Dated: 11/12/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Shixin Qin, Walter Newman and Nasim Kassam		
Application No.:	09/960,547	Group Art Unit:	1646
Filed:	September 21, 2001	Examiner:	Ulm, John D.
Confirmation No.:	6606		
Title:	ANTI-CCR1 ANTIBODIES AND METHODS OF USE THEREFOR		

CERTIFICATE OF MAILING OR TRANSMISSION

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, or is being facsimile transmitted to the United States Patent and Trademark Office on:

11/12/03 Paula DePeltteau
Date Signature

PAULA DEPELTEAU
Typed or printed name of person signing certificate

STATEMENT UNDER 37 C.F.R. §1.806 and §1.808

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Pursuant to 37 C.F.R. §1.806 and §1.808, the undersigned attorney states as follows:

1. The above-referenced application contains reference to a biological deposit deposited under the Budapest Treaty at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209. Murine hybridoma 2D4 (LS-125-2D4-11-10-1) was deposited on February 1, 1999, and assigned ATCC Accession Number HB-12644. A copy of the ATCC Deposit Receipt for this deposit is attached as Exhibit 1.

2. Deposit HB-12644 will be maintained in a public depository for the enforceable life of the patent which issues from the above-referenced application, a term of at least thirty years from the date of deposit or at least five years after the most recent request for the furnishing of a sample of the deposit is received by the depository, whichever is longer.

3. In accordance with 37 C.F.R. §1.808(a)(1), access to deposit HB-12644 will be available during the pendency of the above-referenced application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122.

4. In accordance with 37 C.F.R. §1.808(a)(2), and except as permitted by 37 C.F.R. §1.808(b), all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent on the above-referenced application.

5. The undersigned is an attorney of record.

Respectfully submitted,
HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Lisa M. Treannie

Lisa M. Treannie
Registration No. 41,368
Telephone (978) 341-0036
Facsimile (978) 341-0136

Concord, MA 01742-9133
Dated: 11/12/03

ATCC

CORRECTED

COPY

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

LeukoSite, Inc.
Attn: Shixin Qin
215 First Street
Cambridge, MA 02142

Deposited on Behalf of: LeukoSite, Inc.

Identification Reference by Depositor:	**ATCC Designation**
Mouse hybridoma LS-125-2D4-11-10-1	HB-12644

The deposit was accompanied by: ___ a scientific description _ a proposed taxonomic description indicated above.

The deposit was received February 1, 1999 by this International Depository Authority and has been accepted.

AT YOUR REQUEST: X We will not inform you of requests for the strain.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested February 23, 1999. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Barbara M. Hailey

Barbara M. Hailey, Administrator, Patent Deposit ry

Date: February 24, 1999

cc: **David E. Brook, Esq. (Ref. LKS97-13)**

A:\416750_1.WPD
LMT/pdd
November 4, 2003

PATENT APPLICATION
Attorney's Docket No.: 1855.1048-010

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Shixin Qin, Walter Newman and Nasim Kassam		
Application No.:	09/960,547	Group Art Unit:	1646
Filed:	September 21, 2001	Examiner:	Ulm, John D.
Confirmation No.:	6606		

Title: ~~ANTI-CCR1 ANTIBODIES AND METHODS OF USE THEREFOR~~

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, P.O. Box 2327, Arlington, VA 22202

on 11/12/03 (Date) Paula Depelteau (Signature)

PAULA DEPELTEAU
Typed or printed name of person signing certificate

DECLARATION UNDER 37 C.F.R. §1.132

Box AF
Assistant Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202

Sir:

I, Dominic Picarella, of 28 Deer Pond Drive, Sudbury, Massachusetts 01776, declare and state as follows:

1. I am currently employed by Millennium Pharmaceuticals, Inc. as a Senior Scientist II and am trained as an immunologist. My curriculum vitae is attached as Exhibit A.

2. The above-identified patent application was filed on behalf of Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, Massachusetts 02139, the Assignee of record. This patent application is a divisional application of U.S. Application Serial No. 09/239,938, filed January 29, 1999.

3. I am familiar with the invention claimed in Claims 1-163 of the above-captioned patent application and have reviewed the rejection of these claims under 35 U.S.C. §112, first paragraph, set forth in the Office Action mailed from the U.S. Patent and Trademark Office on June 11, 2003. The Examiner states that Claims 1-163 contain subject matter which was not described in the application in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner states that the Specification does not provide the guidance needed to predictably administer an anti-CCR1 antibody to an organism for clinical effect. The Examiner further states that the Specification appears to be relying on procedures "well known to the skilled artisan" to provide the details of the claimed method and alleges that the administration of antibodies for clinical effect does not appear to have been well known to the skilled artisan at the time the application was filed. It is my understanding that Claims 98-163 are being cancelled in the concurrently filed Amendment.

4. At the effective filing date of the above-identified application (January 29, 1999), a large number of monoclonal antibody therapeutics were in clinical use and preclinical trials. This is evidenced by Reichert, *Nature Biotechnology 19*:819-822 (2001) attached as Exhibit B. Reichert provides a review of various aspects of the clinical development of antibody products and notes that between 1980 and 2000, 227 monoclonal antibody products entered commercially sponsored clinical studies. Thus, there was a large body of knowledge available to the skilled artisan in the field of antibody technology, particularly related to the formulation and optimization of antibody products for *in vivo* use, at the effective filing date of the application.

5. The above-identified application discloses antibodies and antigen-binding fragments that bind CCR1 and inhibit binding of a ligand of CCR1 to the receptor. In addition, the application demonstrates that monoclonal antibody 2D4, which binds the second extracellular loop of CCR1, inhibits binding of MIP-1α and RANTES to CCR1 and inhibits MIP-1α- and RANTES-induced chemotaxis of monocytes and calcium flux in monocytes. Given this disclosure, it would have required only routine experimentation for the skilled artisan to make and use the claimed invention. In particular, the skilled artisan would have determined the pharmacodynamics profile *in vitro* (*i.e.*, the amount of antibody needed to completely inhibit, *e.g.*, chemotaxis, and the amount of receptor saturation necessary to achieve complete inhibition) and *in vivo* (*i.e.*, the dose of antibody to be administered to achieve *in vivo* the level of receptor saturation determined *in vitro*). The skilled artisan would also have determined the pK profile (*i.e.*, the concentration of antibody in the blood over time resulting from various amounts of administered antibody) of the antibody to make and use the invention commensurate with the current claims. The procedures for determining both the pharmacodynamics profile and the pK profile were routine in the art at the effective filing date of the application and would not have required undue experimentation.

6. To determine the inhibition activity profile, it was routine to utilize an increasing concentration of antibody in *in vitro* chemotaxis assays to determine the saturation profile of the antibody (% of CCR1 receptors on cells which are occupied by antibody) which results in a particular level (e.g., 90%) of inhibition of chemotaxis. Chemotaxis assays are disclosed in the above-identified application and were routine in the art at the effective filing date of the application.

7. To determine the pK profile, it was routine to use standard dose escalation protocols in which increasing doses of the antibody or antigen-binding fragment are administered and the physiological effects and blood levels of the antibody are monitored over a period of time. CCR1 in particular is easy to work with in this

context because it is an abundant receptor in blood by virtue of its expression on peripheral blood cells.

8. In view of paragraphs 4 through 7 above, it was routine and would not have required undue experimentation for one of skill in the art to determine the route and duration of administration and the quantity of antibody or antigen-binding fragment to be administered to effect the methods claimed in Claims 1-97 at the effective filing date of the above-identified application. Thus, it was well within the ability of the skilled artisan to administer an anti-CCR1 antibody or antigen-binding fragment thereof as disclosed in the above-identified application and recited in the claims to inhibit a CCR1-mediated effect, *i.e.*, to inhibit interaction of a cell bearing CCR1 with a ligand of CCR1, to inhibit a function associated with binding of a chemokine to a mammalian CCR1, or to inhibit leukocyte trafficking, without undue experimentation.

I further declare that all statements made herein of my own knowledge are true and that all statements based on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States code and that such willful and false statements may jeopardize the validity of the application or any patent issuing thereon.

Dominic Picarella — 11/11/03

Dominic Picarella — Date

Dominic Picarella
28 Deer Pond Road
Sudbury, MA 01776
Home Phone (978) 443-0974
Email depic@earthlink.net

EDUCATION

1989-1993 Post Doctoral Fellowship
Department of Immunobiology (Richard Flavell)
Yale University, New Haven CT

1981-1989 Ph.D., Immunology
Sackler School of Biomedical Sciences (Naomi Rosenberg)
Tufts University, Boston MA

1975-1978 M.S., Microbiology
Waksman Institute of Microbiology (Otto Plescia)
Rutgers University, New Brunswick NJ

1971-1975 B.A., Chemistry
Rutgers University, New Brunswick NJ

EMPLOYMENT

2001-present *Senior Scientist II,Inflammation Pharmacology*
Millennium Pharmaceuticals, Inc.
Responsible for the development and implementation of a variety of rodent models of human disease to provide the biological validation of potential therapeutic targets and to provide the pharmacologic validation necessary to advance early development candidates into the clinic

1999-2001 *Senior Scientist I, Experimental Medicine, Millennium Pharmaceuticals, Inc.*
Primary responsibility for in vivo validation effort for new therapeutic targets in Rheumatoid Arthritis & Inflammatory Bowel Disease.
Development of monoclonal antibodies for validation of targets in animal models of disease.
Program leader for CCR6 Discovery

1998-1999 *Senior Scientist II, Immunobiology, LeukoSite Inc.*
Program leader αeβ7 Discovery
Biology leader (validation/small molecule) α4β7 Discovery
Development of monoclonal antibodies for validation of targets

EXHIBIT
A

in animal models of disease

1994-1998 *Senior Research Scientist, Experimental Therapeutics, LeukoSite, Inc.*

Acting Director, Experimental Therapeutics
Managed group of 7 senior scientists and scientists
Directed in vivo validation and pharmacology efforts for 6 drug discovery programs
Biology leader (validation/small molecule) for CCR3 Discovery.

1994-1998 *Senior Scientist, Experimental Therapeutics, LeukoSite, Inc.*

Successfully validated α4β7 as a therapeutic target for inflammatory bowel disease using murine models of colitis
Successfully developed cell-based integrin-soluble ligand binding assay to identify small molecule inhibitors of MAdCAM-1 binding to α4β7
Successfully developed ELISA to detect soluble MAdCAM-1 in blood for use as a biomarker for disease severity and efficacy

1994 *Research Investigator, Department of Pulmonary Medicine, Yale University.*

Established research effort to study the role of cytokines in lung inflammation and pulmonary disease using transgenic mice as models.

1978-1981 *Associate Research Scientist, Wampole Division, Carter-Wallace, Inc.*

Development of next generation of RIA for hCG for pregnancy testing (Beta-Tec)
Supervision of Manufacturing and Quality Control for RIA kit
Development of manufacturing batch records for RIA kit

PUBLICATIONS

Sanchez-Fueyo A, Tian J, **Picarella** D, Domenig C, Zheng XX, Sabatos CA, Manlongat N, Bender O, Kamradt T, Kuchroo VK, Gutierrez-Ramos JC, Coyle AJ, Strom TB. (2003). Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. Nat Immunol. 4:1093-1101.

Lugering A, Kucharzik T, Soler D, **Picarella** D, Hudson JT 3rd, Williams IR. (2003). Lymphoid precursors in intestinal cryptopatches express CCR6 and undergo dysregulated development in the absence of CCR6. J Immunol. 171:2208-2215.

Skak K, Guerder S, **Picarella** DE, Brenden N, Flavell RA, Michelsen BK. (2003). TNF-alpha impairs peripheral tolerance towards beta-cells, and local costimulation by B7.1 enhances the effector function of diabetogenic T cells. Eur J Immunol. 33:1341-1350.

Grant EP, **Picarella** D, Burwell T, Delaney T, Croci A, Avitahl N, Humbles AA, Gutierrez-Ramos JC, Briskin M, Gerard C, Coyle AJ. (2002). Essential role for the C5a receptor in regulating the effector phase of synovial infiltration and joint destruction in experimental arthritis. J Exp Med. 196:1461-1467.

Grewal IS, Grewal KD, Wong FS, Wang H, **Picarella** DE, Janeway CA Jr, Flavell RA. (2002). Expression of transgene encoded TGF-beta in islets prevents autoimmune diabetes in NOD mice by a local mechanism.
J Autoimmun. 19:9-22.

Bowman, E. P., Campbell, J. J., Soler, D., Dong, Z., Manlongat, N., **Picarella**, D. E., and Butcher, E. C. (2000). Developmental switches in chemokine response profiles during B-cell differentiation and maturation. J. Exp. Med. 191:1303-1318.

Picarella, D., Hurlbut, P., Rottman, J., Shi, X., Butcher, E., and Ringler, D.J. (1997) Monoclonal antibodies specific for β7 integrin and mucosal addressin cell adhesion molecule-1 (MAdCAM-1) reduce inflammation in the colon of scid mice reconstituted with CD45RBhigh CD4+ T cells. J. Immunol. 158:2099-2106.

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Rankin, J. A., **Picarella**, D. E., Geba, G. P., Temann, U. A., Prasad, B., DiCosmo, B., Tarallo, A., Stripp, B., Whitsett, J., and Flavell, R. A. (1996). Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. Proc. Natl. Acad. Sci. USA 93:7821-7825.

DiCosmo, B. F., Geba, G. P., **Picar lla**, D. E., Elias, J. A., Rankin, J. A., Stripp, B. R., Whitsett, J., and Flavell, R. A. (1994). Airway

epithelial cell expression of interleukin-6 in transgenic mice. Uncoupling of airway inflammation and bronhial hypereactivity. J. Clin. Invest. 94:2028-2035.

DiCosmo, B. F., **Picarella**, D., and Flavell, R. A. (1994). Local production of human IL-6 promotes insulitis but retards the onset of insulin-dependent diabetes mellitus in non-obese diabetic mice. Int. Immunol. 6:1829-1837.

Guerder, S., **Picarella**, D. E., Linsley, P.S., and Flavell, R. A. (1994). Costimulator B7 confers APC function to parenchymal tissue and, in conjuction with TNF, leads to autoimmunity in transgenic mice. Proc. Natl. Acad. Sci. 91:5138-5142.

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Picarella, D. E., Kratz, A., Li, C-b., Ruddle, N. H., and Flavell, R. A. (1993). Transgenic TNF-a production in pancreatic islets leads to insulitis, not diabetes: distinct patterns of inflammation in TNF-α and TNF-β transgenic mice. *J. Immunology* 150: 4136-4150.

Picarella, D. E., Kratz, A., Li, C-b., Ruddle, N. H., and Flavell, R. A. (1992). Insulitis in transgenic mice expressing tumor necrosis factor β (lymphotoxin) in the pancreas. *Proc. Natl. Acad. Sci.* 89:10036-10040.

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Monoclonal antibodies in the clinic

Despite initial teething problems, the number of clinically effective monoclonal antibodies is growing.

Janice M. Reichert

Monoclonal antibodies (mAbs) are nature's biological warheads, able to target and help eliminate foreign or abnormal agents from the body. In theory, replicating this powerful defense system could cure some of humanity's most deadly diseases. Indeed, when these biologics first entered clinical studies in the early 1980s they were heralded as "magic bullets" for the treatment of cancers, able to seek out and destroy tumor cells. However, early studies were disappointing and since then mAbs have fallen in and out of fashion. Today, 10 mAbs are now approved as therapeutics in the United States, so some of the original enthusiasm appears to have been justified. Here, a retrospective study provides a picture of the success of mAbs in the clinic, and prompts speculation about the most suitable choice of mAb class to pursue as a therapeutic.

Hybridomas were the original source of monoclonal antibodies for clinical trials.

The monoclonal revolution

The first generation of mAbs, unveiled in 1975 (ref. 1), were murine mAbs derived from mouse B-cell hybridomas (see "Monoclonal antibodies by design"). However, as murine mAbs moved into clinical trials, it became clear that they had limited potential as therapeutics. The human immune system recognizes murine mAbs as foreign material, producing human anti-mouse antibodies (HAMAs) to clear them from the body, thereby limiting their therapeutic benefit. Furthermore, murine mAbs are inefficient at triggering the function of effector cells (e.g., macrophages, T cells) needed to eliminate the antibody–antigen complex.

In sum, murine mAbs were disappointing therapeutics, and only one reached the US market. After a two-year review period, the US Food and Drug Administration (FDA) approved muromonab-CD3 in 1986 for the reversal of acute rejection of kidney transplants. However, muromonab's success was dependent, in part, on the fact that patients undergoing kidney transplant are treated with other immunosuppressants and are less prone to HAMA reactions.

If mAbs were to become a class of successful therapeutics, then researchers needed to create nonimmunogenic mAbs with high binding affinities that could trigger the appropriate effectors. The most obvious strategy was to make mAbs more "humanlike" by creating mAbs with human protein sequences. Human hybridomas were produced using the same methods applied to create murine hybridomas[2]. However, the human B-cell lines were unstable, yielding only small amounts of the desired antibody, and few fully human mAbs entered clinical trials during the late 1980s.

Subsequent efforts concentrated on using genetic manipulation to produce human or humanlike monoclonal products. Chimeric mAbs, specifically human–murine hybrids (see "Monoclonal antibodies by design"), were described in several publications during 1984 (refs 3,4), and the first entered clinical trials in 1987. Researchers hoped that the human constant regions of the mAbs would not only

Table 1. Success rates for mAbs entering clinical trials

Initiation of clinical trials (years)	Total number of mAbs	Number of mAbs discontinued	Number of mAbs approved	% completion[a]	% success[b]
1980–1982	2	1	1	100	50
1983–1985	9	8	0	89	0
1986–1988	33	29	2	94	6
1989–1991	34	29	2	91	6
1992–1994	41	23	5	68	18
1995–1997	33	12	0	36	0
1998–2000	34	2	0	6	0
All mAbs (1980–2000)	186	104	10	61	9
Murine mAbs	49	34	1	71	3
Chimeric mAbs	23	13	4	74	24
Humanized mAbs	59	15	5	34	25

[a] % completion = the percentage of products that have been discontinued and approved, providing an indication of how far trials have progressed. A low value will inevitably reduce the accuracy of the estimated success rates for that class of mAbs.
[b] % success = the percentage of mAbs that successfully completed trials and were approved by the US FDA.

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EXHIBIT B

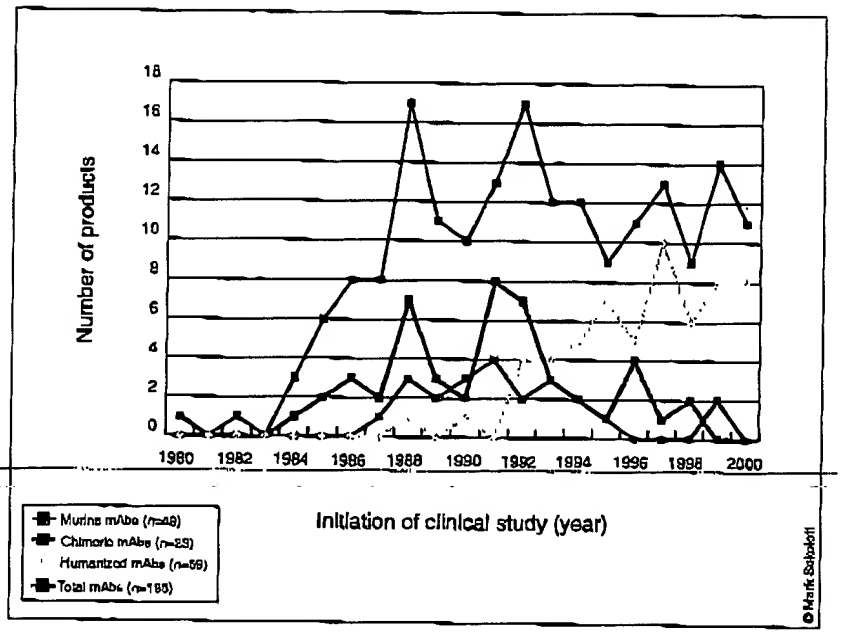

Figure 1. Number of mAbs entering clinical studies by year. Total mAbs includes murine, chimeric, humanized, Primatized, human, and mAbs of unidentified category.

trigger effector functions, but also make the products invisible to the patient's immune system, thereby eliminating the HAMA response. On the assumption that a greater percentage of native human sequence would provide a more effective therapeutic, humanized mAbs were produced in 1986 (ref. 5), and the first commercially sponsored humanized mAb entered clinical studies in 1988.

Into the clinic

Between 1980 and 1992, murine mAbs were the predominant mAbs entering clinical studies, reaching a "peak" of eight products during the year 1991 (see Fig. 1). Chimeric mAbs were less evident: Between two and four products entered clinical studies each year during 1988–1994. By comparison, although few humanized mAbs entered clinical studies during the late 1980s and early 1990s, this class of mAbs has been the main source of antibody-based products entering clinical studies since 1997.

Although production of fully human mAbs from transgenic mice and phage display has been possible since the early 1990s, disputes over the patents for the technologies has delayed the investigation of this class of mAbs. In particular, the dispute between GenPharm (now Medarex of Princeton, NJ) and Cell Genesys (now Abgenix of Fremont, CA) was not resolved until 1997 (through a cross-licensing agreement), and there is ongoing litigation between MorphoSys (Martinsried, Germany) and Cambridge Antibody Technology (Cambridge, UK) over antibody phage display technology. Nevertheless, despite scientific and legal issues, the biopharmaceutical industry has embraced the notion that the more "humanlike" the mAb, the greater its chance of being a safe and effective medicine. To determine whether or not this preference may be justified, we studied the difference in success rates for the different categories of mAbs.

We collected clinical development and regulatory review data for 186 products entering clinical studies by United States–based commercial sponsors during 1980–2000 (see "Analysis criteria"). The data were classed by the year that the clinical studies were initiated, and by the specific mAb category (murine, chimeric, or humanized). Success rates were then calculated, being most accurate for the groups with the greatest number of products with known fates. Note that the success rates calculated for the 1995–1997 and 1998–2000 periods are less accurate: On average, approved mAbs spent 63.1 months[6] in clinical development and regulatory review, and so insufficient time has passed for accurate estimates to be made.

After an initial high success rate of 50%—a value that was skewed by the success of muromonab—the success rates for mAbs dropped over time, not rising to double-digit figures (18%) again until 1992–1994 (Table 1). During that period, a larger number of humanized mAbs began entering clinical studies.

Monoclonal antibodies by design

Antibody: Complex protein-based molecules produced by B lymphocytes that bind to and help eliminate foreign and infectious agents in the body. Antibodies are Y-shaped, having two sets of branches attached to a single stem. The arms of the Y are the so-called variable regions, the tips of the arms contain antigen-binding regions, and the stem is a constant region. The constant region triggers effector functions (phagocytosis, cytolysis, or initiation of complement cascade followed by cell lysis) by linking the complex to other cells of the immune system.

Monoclonal antibody (mAb): Originally, mAbs were antibodies produced from a single B lymphocyte. Genetic manipulation now allows genes from multiple sources of B lymphocytes (e.g., mouse and human) to be combined. Monoclonal antibodies of a defined peptide sequence have identical antigen-binding regions and bind to the same site (the epitope) of an antigen.

Murine (mouse) mAb: A mAb derived entirely from mice, specifically murine hybridomas generated from the fusion of mouse myeloma and mouse B-lymphocyte cells.

Chimeric mAb: A mAb constructed from variable regions derived from murine source and constant regions derived from a human source.

Humanized mAb: A mAb constructed with only antigen-binding regions (also called complementarity-determining regions, or CDRs) derived from a mouse, and the remainder of the variable regions, and constant regions, derived from a human source.

Primatized: A mAb constructed from variable regions derived from *Cynamolgus* macaques and constant regions derived from a human source. Primatized is a registered US trademark of IDEC Pharmaceuticals (San Diego, CA).

Human mAb: A mAb derived entirely from human sources, currently transgenic mice or phage display. Human mAbs can also be produced from human hybridomas or human B-lymphocyte cell lines immortalized by Epstein–Barr virus. However, these cell lines are unstable and produce only small amounts of mAbs. *JR*

Analysis criteria

The biopharmaceuticals database of the Tufts Center for the Study of Drug Development includes data on 227 monoclonal antibody products (92 of which are currently in development) that entered commercially sponsored clinical studies between 1980 and 2000. Records for these products were updated through to mid-May 2001. Data were collected from surveys of sponsoring companies, and from public documents. Data for the 186 mAbs sponsored by United States–based companies were included in the calculation of success rates.

Of the 186 mAbs, 72 were in development (18 were in phase 1, 37 were in phase 2, 14 were at phase 3, and 3 were undergoing regulatory review by the FDA). For calculations of phase transition success rates, phase 1/2 studies were assigned as phase 2, and phase 2/3 studies were assigned as phase 3. Description of the category of mAb (murine, chimeric, humanized, Primatized, or human), which was available for 78% of the mAb products, was either provided by the sponsoring company or assigned from information in public documents. Success rates were not calculated for Primatized or human mAbs because too few have entered clinical studies for any calculated value to be meaningful. *JR*

The average success rate for all mAb products was 9%, but this masks distinct differences in success rates for different categories. Murine mAbs have been the least successful of the mAb products with a success rate of only 3%—not surprising given the scientific and clinical concerns associated with these products. Chimeric and humanized mAbs have higher success rates of 21% and 25%, respectively.

We may predict that the success rates for the humanized mAb group will be higher in the future. The calculated success rate for humanized mAbs presented here is based on just 34% of the total; the rest of the group are still progressing through clinical trials, and their fate is not yet known. Furthermore, in general, the success rate for a class of therapeutic medicines increases as clinical experience of these products accumulates over time[7]. Nevertheless, the success rates for chimeric and humanized mAbs are currently slightly higher than those for new chemical entities (NCEs), ranging from 23.2% during the 1981–1983 period to 17.2% during 1990–1992 (ref. 7).

Making the transition

The probabilities of progressing through clinical development and regulatory review were calculated for the mAb products according to the year that the clinical study was initiated and by the specific category of mAb (Table 2). The probability of mAbs moving from phase 1 to 2 ranged from 50% to 90% during the period 1980–2000. There were lower rates of success for products making the transition between phase 2 and 3 (range 40–55%), and between the phase 3 and the FDA review phase (range 29–67%; the 100% success rate for 1980–1982 was an anomaly because one of two products included in that time period was ultimately approved).

All three categories of mAbs had similar phase 1 to phase 2 transition probabilities. Humanized mAbs had the highest probability of completing phase 2 and entering phase 3, so a higher percentage were effective as therapeutics. However, a clear distinction between the product types emerged after phase 3: Murine mAbs were less likely to go to regulatory review than chimeric and humanized mAbs and were less likely to be approved. Chimeric and humanized mAbs had similar probabilities of making phase transitions from phase 3 to regulatory review, and all were subsequently approved. The perfect score for this latter transition suggests that sponsoring companies applied stringent standards to the clinical data submitted to the FDA—a practice that must continue if this high transition rate is to be maintained.

In comparison, the success rate for NCEs for the transition between phase 1 and 2 is 73%, between phase 2 and 3 is 45%, and between phase 3 and approval is 73% (ref. 7; phase 3 to approval transition rates are 15% for murine mAbs, 80% for chimeric mAbs, and 75% for humanized mAbs). These data suggest that mAbs and NCEs are subject to the same challenges in the clinic. Failures at phase 1, when safety is assessed, should be minimal but may still arise because of unexpected side effects or poor pharmacokinetics and pharmacodynamics not anticipated from preclinical studies. The success rate falls during the transition from phase 2 to 3 because effectiveness is a more difficult standard to achieve. Assuming that only safe and effective candidates move into phase 3, then only unexpected problems, which can happen when clinical studies are scaled up, should derail products at that point. Review to approval success rates should be 100%, as, ideally, companies are presenting unequivocal data to the US FDA.

Monoclonals to the market

Analysis of the success rates of mAbs show that, despite early failures of murine products, chimeric and humanized mAbs are viable therapeutics. There are currently 10 mAb products on the market in the United States (one murine, four chimeric, and five humanized), and more than 70 products in clinical development (see Table 3). We can use the success rates here to predict that 10–12 of these chimeric and humanized mAbs products in development will be approved for marketing within the next five to six years. Humanized mAbs may have an even higher success rate than was calculated from the data currently available, and in this case an even larger number of products may gain approval.

The industry has the technologies needed to optimize both the specificity and yield of mAbs, providing products that are

Table 2. Phase transition probabilities for mAbs

Initiation of clinical study (year)	% completion[a]	Phase 1 to Phase 2[b]	Phase 2 to Phase 3[b]	Phase 3 to Review[b]	Review to Approval[b]
1980–1982	100	50%	100%	100%	100%
1983–1985	89	67%	50%	50%	0%
1986–1988	94	58%	47%	57%	50%
1989–1991	91	64%	40%	29%	100%
1992–1994	68	85%	55%	67%	100%
1995–1997	38	77%	55%	NA	NA
1998–2000	6	80%	NA	NA	NA
Murine mAbs	71	77%	52%	45%	33%
Chimeric mAbs	74	86%	40%	80%	100%
Humanized mAbs	34	84%	72%	75%	100%

[a] % completion = see Fig. 1

[b] Success rates for phase transitions were calculated as follow: the number of products that completed a given phase (e.g., phase 1) and entered the next phase (e.g., phase 2), divided by the total number of products that entered the first phase and did not remain in that phase (i.e., all products entering the phase minus those that remained).

NA = Not applicable; calculation required division by zero.

Table 3. Therapeutic monoclonal antibodies approved by the US FDA

Generic name	Trade name	Sponsor company	Type	Approval date
Muromonab-CD3	Orthoclone	Ortho Biotech	Murine	1986
Abciximab	ReoPro	Centocor	Chimeric	1994
Rituximab	Rituxan	Genentech	Chimeric	1997
Daclizumab	Zenapax	Hoffman-La Roche	Humanized	1997
Basiliximab	Simulect	Novartis	Chimeric	1998
Palivizumab	Synagis	MedImmune	Humanized	1998
Infliximab	Remicade	Centocor	Chimeric	1998
Trastuzumab	Herceptin	Genentech	Humanized	1998
Gemtuzumab ozogamicin	Mylotarg	Wyeth-Ayerst	Humanized	2000
Alemtuzumab	Campath	Millennium/ILEX	Humanized	2001

clinically effective and cost effective to manufacture. Production methods depend on the type of mAb desired: Chimeric or humanized mAbs can be made by genetic manipulation and produced in mammalian, plant, or insect cells; human mAbs can be produced by phage display, or from transgenic animals such as mice and goats. The complexity of the production using any of these methods is not very different from that used for other recombinant protein products. However, given the current concerns about the deficits in manufacturing capacity for biopharmaceuticals[8], where will these products be produced? The pharmaceutical and financial industries, and governments, may need to work together to address the long-term planning and investment issues associated with building new manufacturing facilities for such products.

The technology used to produce mAbs will continue to evolve. Human mAbs were not included in this study because few have, as yet, entered clinical studies, although this situation will change in the near future. How this will change the landscape for mAbs remains to be seen. Will the increasing numbers of human mAbs eclipse humanized mAbs? Will the human mAbs have a higher success rate than chimeric or humanized mAbs? Regardless of the answers to these questions, it is clear that therapeutic mAbs have proved their worth and are here to stay. Therapeutic mAbs have unique characteristics that cannot be duplicated by small-molecule drugs, and are welcome additions to the armory of therapeutic medicines.

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